ULTRAVIOLET ABSORPTION STUDIES ON TOBACCO MOSAIC VIRUS NUCLEIC ACID*

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Nucleic acid has a characteristic absorption in the ultraviolet region with a maximum around 260 mμ and this is due to the presence of purine and pyrimidine rings in its molecule. Treatment of ribonucleic acid with various degrading agents such as alkali, ribonuclease and perchloric acid is accompanied by an increase in the absorption at 260 mμ (hyperchromic effect)[1–6]. MAGASANIK AND CHARGAFF[4] attributed this phenomenon to the hindrance of resonance of guanine ring in the highly polymerized molecules. The present communication deals with the effects of alkali, pancreatic ribonuclease and leaf ribonuclease on the absorption of tobacco mosaic virus nucleic acid (TMV-NA) and its ribonuclease resistant-residue, "Core". The results recorded here show that polypurine nucleotide segments of the nucleic acid molecule are mainly responsible for the hyperchromic effect following the degradation of the nucleic acid and that it is the composition and not the size of the molecule that influences this effect.

EXPERIMENTAL

Materials

Nucleic acid from purified TMV was prepared using the heat denaturation method[7,8] with the following modification.

Unless otherwise stated all the operations were done between 0°–4° C. To 1 ml of 0.3 *M* NaCl at 100° C was added 1 ml of TMV (about 25 mg TMV/ml). The mixture was held at this temperature for one minute and then transferred to an ice bath. The contents of several tubes were pooled and centrifuged in a Sorvall centrifuge at 10,000 r.p.m. for 15 minutes. The supernatant was filtered through a wet fluted Whatman No. 1 filter paper to remove the floating denatured protein particles that resisted centrifugation. To this filtrate two volumes of alcohol were added and the mixture was held for one hour. The precipitate, separated by centrifugation, was washed two times with 66% alcohol, then taken up in distilled water (1 ml distilled water for about 10 mg nucleic acid) and centrifuged at 40,000 r.p.m. for two hours. The supernatant was lyophilized. The nucleic acid thus prepared was used in the following studies.

Pancreatic ribonuclease used in these studies was a crystalline preparation obtained from the Worthington Biochemical Corporation, Freehold, New Jersey.

Leaf ribonuclease was prepared from healthy Turkish tobacco leaves according to the procedure of FRISCH-NIGGEMEYER AND REDDI[9].

"Core", pancreatic ribonuclease resistant residue, was prepared according to the method of REDDI AND KNIGHT[10].

Methods

Phosphorus was estimated using the procedure of KING[11] with a slight modification in the digestion[12].

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The ultraviolet absorption measurements were made as follows. An aliquot of the test solution was made up to 10 ml with 0.01 *M* phosphate buffer at pH 7.4 and the absorption was measured in 1 cm quartz cells in a Beckman spectrophotometer Model DU at wave length 260 mμ. The results were expressed as $\varepsilon(P)$[13]. When the measurements were made in distilled water instead of in phosphate buffer, there was a 15% increase in the absorption of nucleic acid at 260 mμ. However, measurements in a buffer medium will have an advantage over those in distilled water, since the former ensures the uniformity of reaction and thus there will be better reproducibility. Hence all the measurements were made in 0.01 *M* phosphate buffer at pH 7.4.

1. *Degradation of nucleic acid with* N *NaOH*. About 3 mg of TMV-NA were dissolved in 1.5 ml distilled water. Phosphorus was determined in an aliquot. 0.1 ml was diluted to 10 ml with 0.01 *M* phosphate buffer and the absorption was measured at 260 mμ. This measurement served as 0 time reading in this experiment, since the contact of NaOH with the nucleic acid brought about an immediate increase in the absorption. To 1 ml TMV-NA in distilled water, 1 ml of 2 *N* NaOH was added and a portion was held at 23° C and another at 37° C. At intervals of time 0.1 ml aliquots were withdrawn and their absorption measured as described above. The results are presented in Fig. 1.

2. *Degradation with leaf ribonuclease*. About 2 mg TMV-NA were dissolved in 2 ml of 0.1 *M* acetate buffer at pH 5.1 and to this was added 0.05 ml leaf ribonuclease (200 units). In an aliquot phosphorus was determined. A portion of this was held at 23° C and another at 37° C. At intervals of time 0.1 ml aliquots were withdrawn and the absorption was measured as described above. The results are given in Fig. 2.

3. *Degradation with pancreatic ribonuclease*. About 2 mg TMV-NA were dissolved in 1.9 ml of 0.1 *M* borate buffer at pH 7.6, and to this 0.1 ml ribonuclease (20 γ) was added. In an aliquot phosphorus was determined. A portion of this was held at 23° C and another portion at 37° C. At intervals of time 0.1 ml aliquots were withdrawn and the absorption was measured as described above. The results are given in Fig. 3.

4. *Degradation of "Core"*. The effects of *N* NaOH and leaf ribonuclease on the absorption of core at 260 mμ were studied in the manner described above for TMV-NA. The results are given in Fig. 4.

RESULTS AND DISCUSSION

When the nucleic acid was exposed to *N* NaOH there was a steady increase in the absorption with time and this attained a maximum value in about 8 hours at 37° C and in 12 hours at 23° C (Fig. 1). The total increase in the absorption was 32.3% OSTER AND GRIMSON[1] reported only an increase of 13% in the absorption following

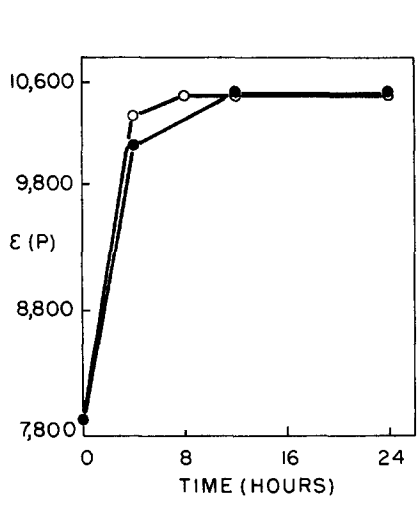

Fig. 1. Effect of *N* NaOH on the absorption of TMV-NA at 260 mμ. O—O 37° C, ●—● 23° C

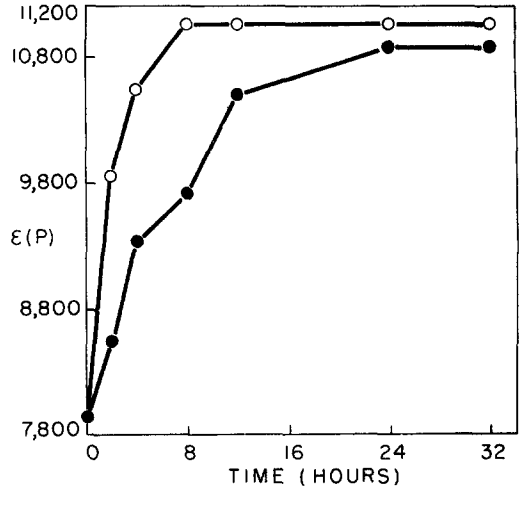

Fig. 2. Effect of leaf ribonuclease on the absorption of TMV-NA at 260 mμ. O—O 37° C, ●—● 23° C

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the treatment of TMV-NA with *N* NaOH, while HOLDEN AND PIRIE[6] observed an increase of 24%.

When the nucleic acid was hydrolyzed with the leaf ribonuclease there was a 37% increase in the absorption (Fig. 2). An increment in the absorption to the same extent was observed by HOLDEN AND PIRIE[6]. The leaf ribonuclease degrades nucleic acid more extensively[6,9] than the pancreatic ribonuclease and does not leave any core behind. Unlike pancreatic ribonuclease it disrupts all bonds between the component nucleotides of ribonucleic acid[14].

When the nucleic acid was hydrolyzed with pancreatic ribonuclease there was about 15% increase in the absorption. The pancreatic ribonuclease is a highly specific phosphodiesterase which hydrolyzes only secondary phosphate esters of pyrimidine ribonucleoside 3'-phosphates[15–17]. The products of hydrolysis consist of a complex mixture and range in size from mono to hexa nucleotides. It appears from the foregoing data that the maximum increase in the absorption was noticed only when all the

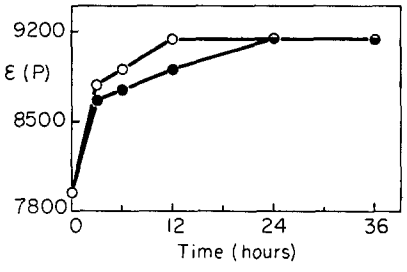

Fig. 3. Effect of pancreatic ribonuclease on the absorption of TMV-NA at 260 mμ. O—O 37° C, ●—● 23° C

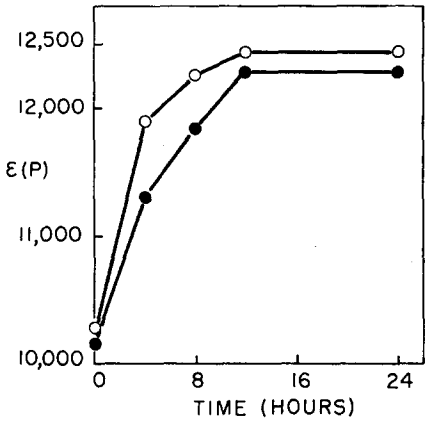

Fig. 4. Effect of *N* NaOH and leaf ribonuclease at 37° C on the absorption of Core at 260 mμ. O—O *N* NaOH, ●—● leaf ribonuclease

bonds between the component nucleotides of nucleic acid were hydrolyzed as in the case of treatment with *N* NaOH or leaf ribonuclease, while no pronounced increase was observed when the cleavage of the molecule was confined to linkages between pyrimidine nucleotides as in the case of treatment with pancreatic ribonuclease.

Following the treatment of pancreatic ribonuclease-resistant residue, "Core" with *N* NaOH or leaf ribonuclease there was an increase in the absorption to about 21% (Fig. 4). Thus the core accounts for about 60% of the total hyperchromic effect of the intact molecule. The core from TMV-NA is rich in adenylic acid and has an average chain length of six nucleotides, five purine nucleotides terminated by a pyrimidine nucleotide[10]. Since the major part of the hyperchromic effect is produced by oligonucleotide segments containing mainly adenine and guanine, it is most likely that the purine part of the nucleic acid is mainly responsible for the increment in the absorption following the degradation. Since the effect is more pronounced in a small fragment, approximately six nucleotides in length, the size does not seem to be the main factor of this effect. Furthermore the core is rich in purines and poor in pyrimi-

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dines, which is in contrast to the composition of the intact polymerized TMV-NA and hence it is likely that the chemical composition and not the size that influences the hyperchromic effect.

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SUMMARY

The effect of alkali, leaf ribonuclease and pancreatic ribonuclease on the changes in absorption of TMV-NA and its core was studied. It was found that alkali and leaf ribonuclease brought about increase in absorption ranging from 32 to 37%, while the pancreatic ribonuclease brought about a 15% increment. The increase in the absorption obtained following the treatment of the core with ribonuclease or *N* NaOH accounts for about 60% of the total increment brought about by the action of *N* NaOH or leaf ribonuclease on the intact TMV-NA. It was concluded that the polypurine nucleotide segments of TMV-NA are responsible for the major increase.

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MECHANISMS OF ANTIBODY GLOBULIN SYNTHESIS BY LYMPHOID TISSUE *IN VITRO**

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In a previous study[1] evidence was presented for *de novo* synthesis of diphtheria antibody when lymphoid tissue from immunized animals was incubated in a suitable medium. The increase in the antibody activity of the system during incubation apparently required intact cells and a source of energy since disruption of the tissue,

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